Queen's University
Belfast

Molecular mechanisms of measles virus persistence.

Published in:
Virus Research

Document Version:
Publisher's PDF, also known as Version of record

Queen's University Belfast - Research Portal:
Link to publication record in Queen's University Belfast Research Portal

ELSEVIER

Review

Molecular mechanisms of measles virus persistence

Bertus K. Rima *, W. Paul Duprex

School of Biology and Biochemistry and Centre for Cancer Research and Cell Biology, The Queen's University of Belfast, Belfast BT9 7BL, Northern Ireland, UK

Available online 12 May 2005

Abstract

As measles virus causes subacute sclerosing panencephalitis and measles inclusion body encephalitis due to its ability to establish human persistent infection, without symptoms for the time between the acute infection and the onset of clinical symptoms, it has been the paradigm for a long term persistent as opposed to chronic infection by an RNA virus. We have reviewed the mechanisms of persistence of the virus and discuss specific mutations associated with CNS infection affecting the matrix and fusion protein genes. These are placed in the context of our current understanding of the viral replication cycle. We also consider the proposed mechanisms of persistence of the virus in replicating cell cultures and conclude that no general mechanistic model can be derived from our current state of knowledge. Finally, we indicate how reverse genetics approaches and the use of mouse models with specific knock-out and knock-in modifications can further our understanding of measles virus persistence.
© 2005 Published by Elsevier B.V.

Keywords: Measles virus; Persistent infection; Subacute sclerosing panencephalitis; Brain receptor; Mechanisms of persistence; Pathogenesis

Contents

* Corresponding author.
E-mail address: b.rima@qub.ac.uk (B.K. Rima).

1. The scope of the review

Measles virus (MV) still provides one of the main paradigms of a long-term persistent infection by an RNA virus due to its involvement in the disease subacute sclerosing panencephalitis (SSPE), a progressive fatal neurological disease caused by high levels of neuronal infection by MV in the central nervous system (CNS). Hence, there has always been an interest in studying the underlying mechanisms of MV persistence. Initially, in the late 1960s and the 1970s, this was studied using cell cultures. However, with the advent in the 1980s and early 1990s of cDNA cloning and sequencing technology it became possible to examine virus genomes present in autopsy material obtained from the CNS of patients. This review will briefly recapitulate the main findings of the earlier studies and will then concentrate on setting out what has been found since the early 1990s. It will illustrate the utility of reverse genetic approaches and transgenic mouse studies and show how these, in the future, may further our understanding of viral persistence in a mechanistic sense. To date no study has been able to explain why the virus is able to persist. As such we use this review to highlight the most pertinent questions which remain to be addressed.

2. Subacute sclerosing panencephalitis and measles inclusion body encephalitis

The main impetus for the study of MV persistence has come from the discovery that MV was the cause of SSPE, a human CNS disease that manifests itself long after the acute infection with the virus. MV infection is associated with neurological complications in a small minority of cases. About 1:1000 cases will suffer post-infection encephalitis, which involves mainly perivascular demyelination (Litvak et al., 1943). This often fatal complication appears to be autoimmune in nature as no virus can be demonstrated in the brain of such patients. SSPE has been reported to occur in 1:300,000 cases. However, more recent data suggests that it is much more prevalent and can follow acute MV infection in 1:10,000 cases (Takasu et al., 2003). On average, symptoms present 8 years after the acute infection, but this ranges from 9 months to 30 years. Acute infection below the age of two is a risk factor in this disease, which, as yet inexplicably predominates in males at a 2.5:1 ratio (Halsey et al., 1980). The disease manifests itself in severe demyelination and profound infection of neurones. In the latter stages of SSPE small numbers of oligodendrocytes, astrocytes and endothelial cells have been shown to be affected (Kirk et al., 1991). This inevitably leads to severe neurological deficits and death of the patient. One of the longest recorded patients carried the persistent infection over three decades before the onset of symptoms. In terms of the duration of the symptomatic period, there is a report of a 52 years old who died 4 years after diagnosis (Tanaka et al., 1987). There is no evidence for a reduction in the cell-mediated immune responses to MV in SSPE patients. Furthermore, antibody responses lead to hyperimmunity with extremely high titres of neutralising antibody in both the serum and cerebrospinal fluid (CSF) of patients. The CSF contains oligoclonal bands specific to MV and it has been demonstrated that the antibodies present in these have undergone affinity maturation (Smith-Jensen et al., 2000).

In immuno-compromised patients, measles can give rise to an additional CNS infection, measles inclusion body encephalitis (MIBE) (ter Meulen et al., 1983). In contrast to SSPE, MIBE is not associated with a hyperimmune antibody response to measles proteins or oligoclonal bands in the CNS. Recently MIBE caused the death of a 13-year-old boy who had been treated for chronic granulomatous disease by stem cell therapy. Neither the patient nor the stem cell donor had apparent recent measles exposure or vaccination and neither had visited a region of the world where MV was endemic (Freeman et al., 2004). The simplest explanation may be persistence of the virus in either the donor or the recipient. Nevertheless repeat exposure cannot be ruled out in this case as the virus is highly infectious.

Other suggested sequelae of MV infection are multiple sclerosis, chronically active autoimmune hepatitis, Paget's disease, otosclerosis, Crohn's disease and autism among many other diseases (e.g. Reddy et al., 1999). However, no confirmed evidence has been presented to substantiate these associations, let alone prove a causative relationship and as such discussion of these falls outside the scope of this review.

3. MV: the virus and its replication

In order to understand what is known of the molecular mechanisms of MV persistence it is first necessary to describe the molecular biology and pathology of MV.

3.1. Virion

MV is an enveloped RNA virus classified in the family *Paramyxoviridae* (Griffin, 2001) in the genus *Morbillivirus*
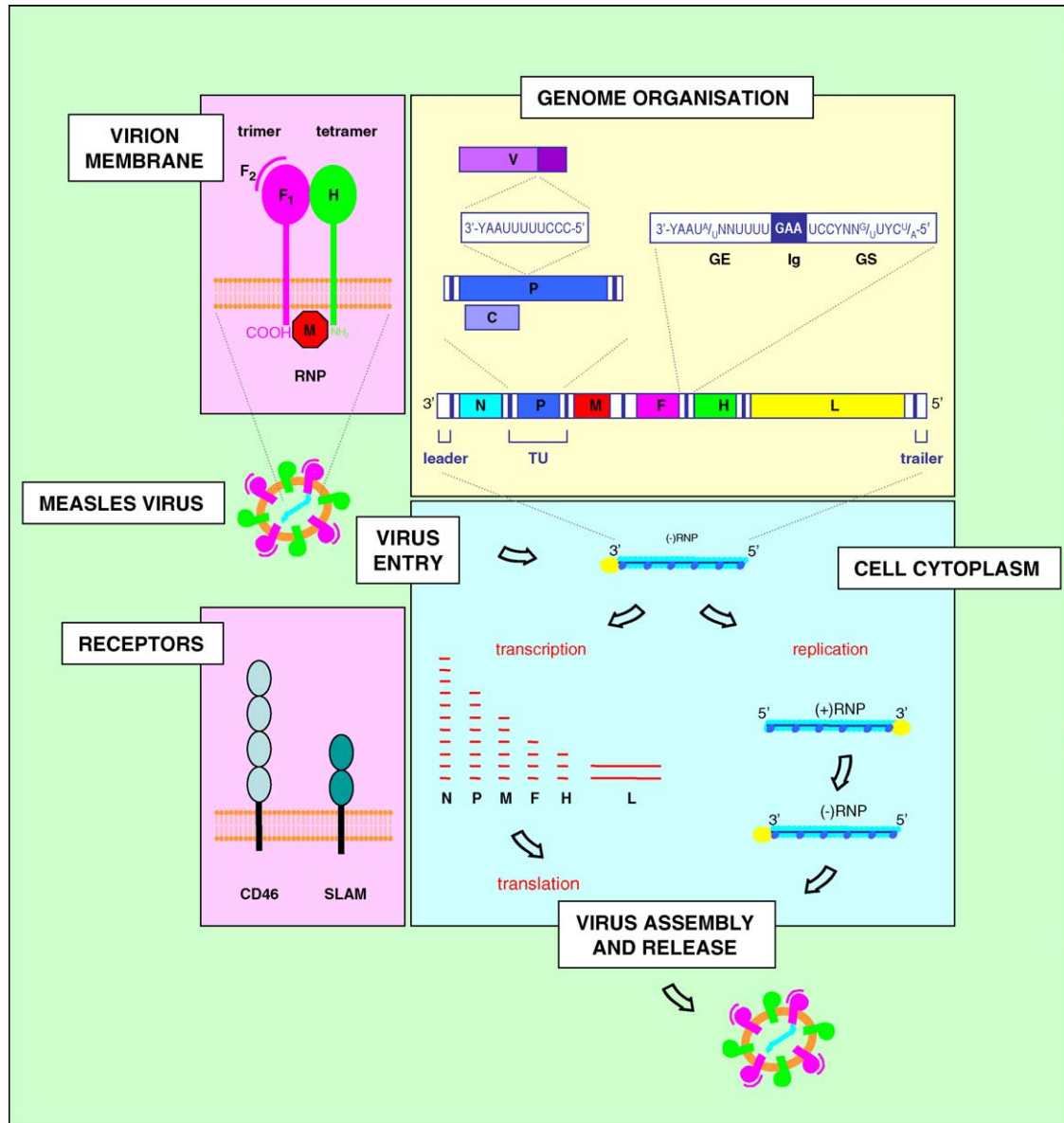
Fig. 1. Schematic representation of the virion membrane, genome organisation and the life-cycle of measles virus including the receptors used by the wild-type and vaccine viruses. Transcription units (TU), and messenger RNAs and proteins are not drawn to scale.

(Fig. 1). The lipid bilayer membrane is derived from the host cell and is decorated with a fringe of spikes formed by two viral glycoproteins, the fusion (F) and the haemagglutinin (H) proteins. In the virion the F (type I) glycoprotein oligomerises as a homotrimer whereas the H (type II) glycoprotein probably exists as a tetrameric dimer-of-dimers. The F protein is formed as an inactive precursor (F_0), which is biologically activated by a proteolytic cleavage involving furin-like proteases (Maisner et al., 2000) to generate the F_1 and F_2 protein subunits, which are linked by a single disulphide bridge. In contrast to some other enveloped viruses, the proteolytic enzymes involved are so widespread that biological activation of the F glycoprotein does not appear to constitute a limiting factor in virus spread. The H and F glycoprotein homo-oligomers interact with each other to form the biologically

active fusion complex (Wild et al., 1991). After binding of the H glycoprotein to cell surface receptor(s), it is likely that a structural change in the F protein is induced which activates the fusion process but the exact stoichiometry and molecular details are unknown. The inner leaflet of the virion membrane is "coated" with a hydrophobic protein called the matrix (M) protein (Fig. 1). This protein associates with both the short cytoplasmic tails of F and H and the helical ribonucleoprotein (RNP) nucleocapsid containing the genome. It plays a role in the budding of the virus from the plasma membrane.

3.2. Genome organisation

Measles virus RNP is 1 μm in length and 18–21 nm in diameter (Lund et al., 1984) and consists of the negative sense

viral genome and at least three associated viral proteins. A recent study has demonstrated that the virions can contain more than one RNP (Rager et al., 2002). The genome is a 15,894 nucleotide, non-segmented RNA molecule of negative polarity (Fig. 1). It contains six transcription units (TUs) which are separated by non-transcribed intergenic (Ig) sequences comprised of three nucleotides. The six TUs are preceded by a 3′ leader and are followed by a 5′ trailer (Fig. 1). Morbillivirus genomes are unique within the *Paramyxoviridae* in that they contain a very long non-coding region of nearly 1000 nucleotides between the M and F open reading frame (ORF). The region is GC rich and it is likely to fold into complex secondary RNA structures. The function of this region is not well understood. The intergenic sequence between the M and F genes is placed almost in the middle of this region.

The complete nucleotide sequence has been determined for several vaccine and wild-type strains of MV. The genome encodes at least eight proteins (Griffin, 2001) (Fig. 1). Three of these, the nucleocapsid (N), phospho- (P) and large (L) proteins, comprise the transcriptionally active RNP. At least two further non-structural proteins are generated from the gene encoding the P protein and as such this gene is sometimes designated P/V/C. The C protein is translated from an overlapping reading frame and is 186 amino acids in length. Whilst the function of the C protein is currently unknown, virus mutants lacking the protein have been propagated successfully in tissue culture (Radecke and Billeter, 1996) but they failed to replicate to a significant level in human thymus implants present in a SCID mouse model and spread less efficiently in a transgenic mouse model (Valsamakis et al., 1998). The C protein is suggested to inhibit the type I interferon response (Shaffer et al., 2003). The V protein is generated from an "edited" transcript of the P gene (Fig. 1). It is amino-co-terminal with the first 231 amino acids of the P protein. Editing involves a process similar to the stuttering on U tracts at the end of each gene which generates polyadenylated mRNAs (see later). Polymerase stuttering at the editing site, 3′-YAAUUUUUCCC-5′ (negative sense), leads to the non-templated insertion of a single G residue in the mRNA (Cattaneo et al., 1989a,b). The consequent frame switch accesses an ORF encoding a 69 residue cysteine-rich tail, which contains a zinc binding domain. This stretch of amino acids represents one of the most conserved products encoded by paramyxovirus genomes.

3.3. Virus entry and cell surface receptors

Measles virus attaches to the host cell through the interaction of the viral glycoproteins with cellular receptors (Fig. 1). The H protein is probably the most important attachment protein but it is likely that the interaction of the complete fusion complex with receptors and possibly co-receptors is required for fusion of the viral envelope with the host cell membrane. CD46, also known as membrane cofactor protein, which plays a role in protecting cells from complement lysis, is a cellular receptor for some laboratory adapted MV vaccine strains (Dörig et al., 1993; Naniche et al., 1993). In cells infected with these MV strains (but not in those infected with wild-type strains), CD46 is down-regulated from the cell surface and this renders the cells more susceptible to complement lysis (Schneider-Schaulies et al., 1995b). Most wild-type viruses do not use CD46 but instead use CD150 (SLAM) as receptor (Erlenhöfer et al., 2001; Tatsuo et al., 2000). Fusion occurs in a pH independent manner at the cell membrane.

3.4. Transcription, replication, assembly and virus budding

After fusion of the virion with the cell membrane, the (−)RNP is introduced into the cytoplasm where it acts as a template for both primary transcription of mRNAs and replication of the negative stranded genome RNA into positive antigenome RNA, which is concomitantly encapsided to produce (+)RNP (Fig. 1). It is probable that the viral RNA-dependent RNA polymerase (RdRp) not only consists of the P and L proteins but it may also involve unknown host cell proteins in addition to cytoskeletal components such as actin and tubulin (Moyer et al., 1990). The RdRp binds to the promoter sequences in the first 100 nucleotides at the 3′ end of the genomic RNA. Transcription terminates just before an intergenic trinucleotide at a gene end (GE) sequence (Fig. 1). For example, at the end of the N gene the RdRp encounters a consensus polyadenylation signal sequence of $3′-YAAU^A/_UNNUUUU-5′$ which leads to polyadenylation of the mRNA by slippage on the U-rich template. The intergenic sequence (3′-GAA-5′) is not transcribed and transcription restarts at the 3′ end of the P/V/C gene at a consensus gene start (GS) sequence of $3′-UCCYNN^G/_UUYC^U/_A-5′$ (Radecke and Billeter, 1995). These stop/start events are repeated at the ends of the P, M, F, H and L genes. There is a finite chance that the RdRp will detach from the template at each intergenic junction and this gives rise to a gradient of gene expression in which the promoter proximal genes (i.e. N and P/V/C) are transcribed much more frequently into mRNA than the promoter distal genes (i.e. H and L).

During replication the nascent RNA molecule is immediately encapsidated by N protein and the GE, Ig trinucleotide and GS signals are ignored. This gives rise to an RNP containing the positive stranded RNA antigenome molecule. These have a strong promoter sequence at their 3′ end, which allows the generation of (−)RNPs. These are transported to the cell membrane where they associate with viral M protein and the glycoproteins in lipid raft structures from which the RNPs bud to form new virus particles.

Development of systems during the last decade which permit the generation of recombinant MV, "virus rescue" (Radecke et al., 1995) has opened the door to mechanistic studies, not only of persistence and the role of specific genes in this process, but also dissection of the roles of individual genes in isolation or in combination with others,

in attenuation, virulence immunosuppression and interaction with the innate immune system.

4. Studies of MV persistence

Persistent infection in tissue culture cells by RNA viruses was a well studied topic in the 1960s and 1970s but became less fashionable, primarily because it was at that stage impossible to provide anything more than a description of very basic culture characteristics such as whether or not the cells produced virus and if so whether the virus had a mutant phenotype. Moreover, the paucity of discernable MV phenotypes hampered these important studies. Only when the viral proteins were delineated and when cloning and sequencing of the viral genome had been completed, was it possible to conceive of studies that would address mechanisms of persistence. Earlier reviews of persistent MV infections in cell cultures (Rima and Martin, 1976) and in the brain (Rima and Martin 1976; Schneider-Schaulies et al., 1995a) postulated roles for viral defective interfering (DI) particles, viral mutants, interferons, etc. However, no conclusive proposals for mechanisms for persistence emerged from these.

5. Persistence in cell culture

In this section and the next we review data obtained from the generation of persistently infected cell cultures and from sequencing material obtained from SSPE cases. What has been learnt from these experimental models of persistence and from clinical material is presented in a unified diagram (Fig. 2) in order to present the key alterations to the virus genome, in the virus life-cycle and to the host cell which have been associated with the persistent state in vitro and in vivo. When reviewing in vitro studies into persistence by RNA viruses it is important to distinguish between "carrier" cultures and "true" persistent infections. In "carrier" cultures uninfected cells are constantly present due to the existence of a dynamic equilibrium between virus infected and uninfected cells, virus release and cell replication in the culture. In a "true" persistent culture all cells are infected. "Carrier" cultures have been considered less relevant as a model for in vivo persistence of MV.

5.1. Generation of persistently-infected, non-neural cell lines

Early studies by Rustigian and co-workers (Rustigian, 1962, 1966a,b; Rustigian et al., 1979) and (Minagawa et al., 1976) demonstrated that it was relatively straightforward to establish "true" persistent infections with MV in tissue culture cells such as HeLa cells (Rustigian, 1962, 1966a,b). Others used Hep2 cells (Gould and Linton, 1975; McCullough, 1983; Rice and Wolff, 1978), monkey kidney cells (Burnstein et al., 1974; Crespi et al., 1988; Hummel et al., 1994; Rima et

al., 1977; Wild and Dugre, 1978) or calf kidney cells (Sorodoc et al., 1980) to produce similar cell lines. Such persistently infected cell lines were initially established using viruses derived from the original Edmonston isolate made by John Enders in 1954. These Edmonston strain derivatives were propagated many times and adapted to laboratory passage and form the basis of all MV vaccines. It was clear that MV could set up persistent infection in many human cell lines such as human amnion AV3 cells (Rapp and Robbins, 1981), leukocytes (Jacobson and McFarland, 1982; Joseph et al., 1975) and lymphoblastoid cells (Celma and Fernandez-Muñoz, 1992; Cremer et al., 1979; Fernandez-Muñoz and Celma, 1992; Ju et al., 1980). In general one can draw the conclusion that such persistent infections allow the accumulation of viral mutants, e.g. temperature sensitive mutants, cold sensitive mutants (Rager-Zisman et al., 1984), small plaque mutants or non-syncytial mutants (McCullough, 1983) as well as mutants that were able to set up persistent infection more readily than the original virus.

It has always been difficult to study the establishment phase as often the cultures did not show a consistent level of infection and underwent crises of cytopathology. This was especially the case in persistent infections in which DI particles had been involved and also in those in which a small number of cells that had survived a lytic infection were cultured (Rima et al., 1977). A number of studies were carried out in the 1970s that dealt with mechanisms of maintenance of stable persistent infections. Rustigian's experiments of co-culture of his HeLa cells with Vero cells to address the nature of the blockage in K11A-HG-1 cells (Rustigian et al., 1979) demonstrated that the HeLa cells were infected by a defective MV which upon introduction into an adjacent Vero cell led to the formation of non-transmissible transient syncytia formation from a very small sub-fraction (0.2–0.4%) of the persistently infected cells. Very occasionally transmissible infections developed after 6–9 weeks from these co-cultures indicating reversion of the virus to an independent fully-infectious agent. Whether this involved back mutations is not known. Recombination was also proposed as a possible mechanism for the reversion, but direct evidence for recombination in non-segmented negative strand RNA viruses is still scant. The co-cultures were transiently resistant to "superinfection" by a second equivalent dose of the K11A-HG-1 cells. However, these studies were limited as superinfection could only be assessed by the formation of an overt cytopathic effect (c.p.e.) such as syncytium formation. Interestingly, to this day the mechanisms involved in superinfection immunity remain elusive. Receptor down-regulation has been put forward an explanation, but usually this is not sufficiently quantitative/complete to explain superinfection immunity on the basis of blocked entry. Furthermore all cells persistently infected with vaccine strains of MV are superinfection immune to vaccine strains of the closely related canine distemper virus which does not use CD46 (Rima, unpublished; Ludlow et al., 2005).
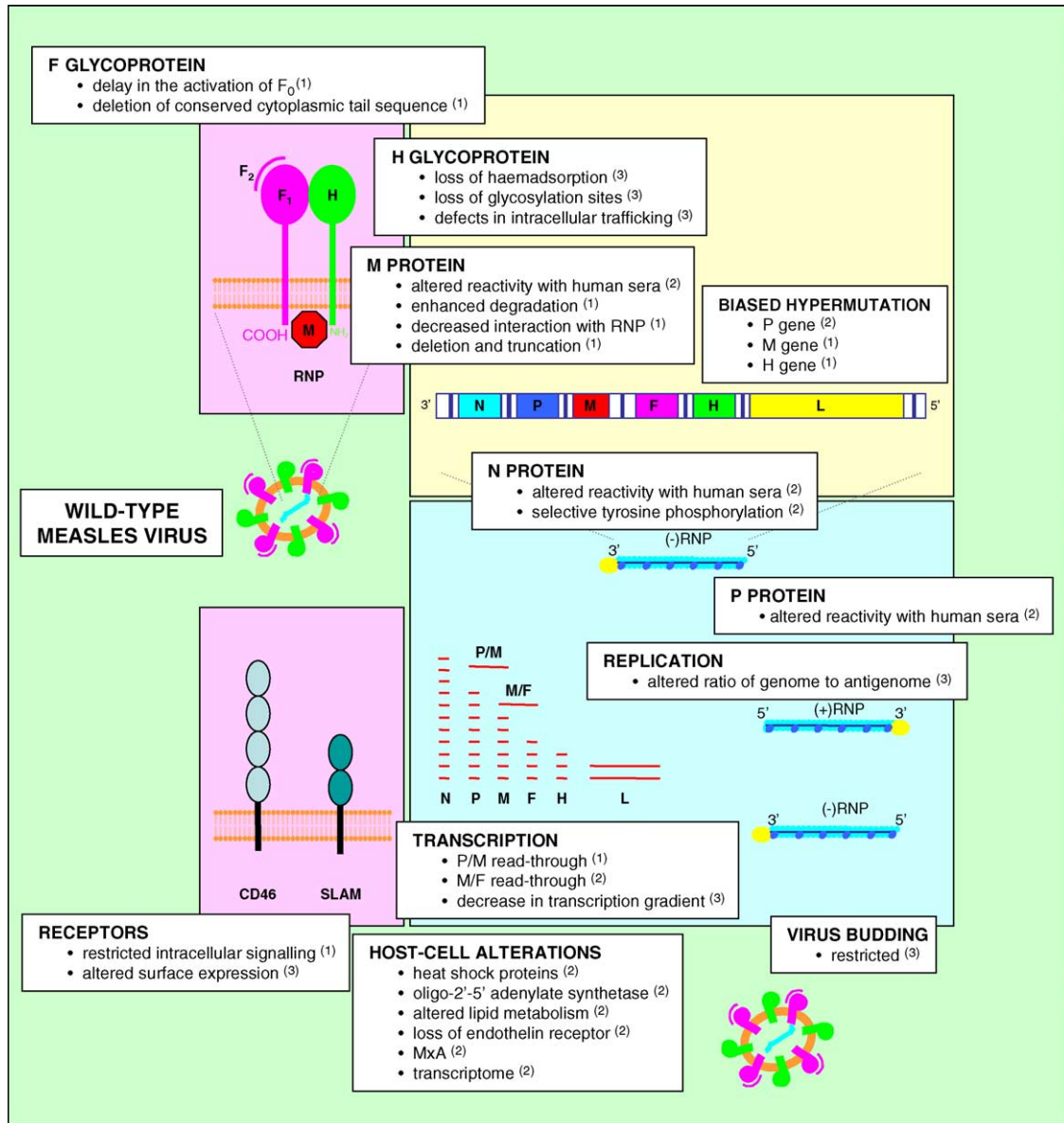
Fig. 2. Compilation of key alterations at the molecular level which have been identified either by sequencing and culturing material derived from SSPE tissue samples (1) or by generating persistently infected neural and non-neural cell lines (2). Those alterations at the molecular level where studies using SSPE tissue samples and persistently infected neural and non-neural cell lines have provided equivalent data have also been indicated (3).

The appearance of mutants in persistently infected cultures was first demonstrated by comparison of serum responses in human convalescent and SSPE sera changes in patterns of antigenicity of the H protein (Robbins et al., 1991), N protein (Boriskin et al., 1986), the P or V protein (Bellini et al., 1986). Reduced amounts of M protein and altered processing of the H protein in persistently infected HeLa cell cultures were shown by difficult pulse-chase experiments (Young et al., 1985). Later the development of monoclonal antibodies to the different MV proteins made it much easier to study mutations and alterations in expression of specific MV proteins in persistence. Due to the lack of the M protein in particular, restriction of viral budding was offered as a mechanism of viral persistence. However, in a persistent infection of Vero cells with the hamster neurotropic (HNT) strain of MV restriction in the expression of the fusion protein was suggested as a mechanism for the maintenance of persistence (Hummel et al., 1994). The lack of F protein expression in these cells was attributed to the overabundance of M/F read-through transcripts (5:1 over F transcripts). Host factors involved in the control of persistent infection have been suggested to relate to elements of innate immunity such as oligo-2′,5′-adenylate synthetase (Fujii et al., 1988, 1990), interaction with heat shock proteins (Oglesbee et al., 1993) or altered lipid metabolism in the cells (Anderton et al., 1981, 1982, 1983a,b; Wild et al., 1981, 1986; Wild and Dugre,

1978). However, in none of these cases has the suggested mechanism been further investigated. Part of the reasons for this may be the "perceived" irrelevance of the study of non-neuronal persistent infection to CNS persistence of MV in SSPE and MIBE. However, these studies may actually be highly pertinent if the site of persistence of MV in SSPE is not in the CNS (see later).

5.2. Generation of persistently-infected, neural cell lines

More effort has been made to establish mechanistic parameters in persistently infected cells of neural origin. Persistent infections have been established in a number of rodent neural cell lines such as hamster brain cells (Vainionpää et al., 1982), in C6 rat brain glioma cells (Halbach and Koschel, 1979; Koschel and Muenzel, 1980) and mouse neuroblastoma cells (Gopas et al., 1992). Mechanistic studies in the C6 rat glioma cells demonstrated a loss of function in the endothelin signalling pathway by loss of its receptor (Meissner and Koschel, 1995; Tas and Koschel, 1991) but this was not functionally linked to the phenomenon of persistence. The Rager–Zisman group established a persistent infection in a C1300 neuroblastoma cell clone called NS20Y (Rager-Zisman et al., 1984). These murine cells produced cold-sensitive mutant viruses and showed an increase in MHC-I expression. They also showed that a tyrosine residue of the N protein was phosphorylated in the persistently infected cells as opposed to in an acute infection, where only serine and threonine are phosphorylated (Segev et al., 1995). Many other aspects of these cells such as transcription regulation; interferon induction and the expression levels of isoforms of protein kinase C were investigated (Fishman et al., 1997; Wolfson et al., 1989). Nevertheless, these studies, which report the most extensive data on a specific MV persistently infected cell line, do not provide a coherent mechanistic explanation for persistence in vitro let alone in vivo. Furthermore the results have not been confirmed by equivalent experiments in other systems, for example persistently infected human neuronal cells, and this makes it at present impossible to use these observations to provide a generalised explanation of MV persistence.

Since rodents are not natural hosts for any of the known morbilliviruses studies of persistence and replication of MV in human cells of neuronal origin are possibly more relevant to in vivo persistence. Sybille Schneider-Schaulies and her colleagues have demonstrated differences in MV transcription and translation in six human glioma cell lines (D-54, D-32, U-251, U-105, U-373 and U-138) (Schneider-Schaulies et al., 1993a,b, 1994). These cells are permissive for MV replication, but overall levels of transcription and replication are lower than in equivalently infected HEL or Vero cells. As mentioned above there is a finite chance that the RdRp will dissociate from the template at each intergenic junction. This gives rise to the transcription gradient, which has been shown to be steeper in rat astroglial cells and in SSPE patient derived material (see below). Lower relative amounts of the M, F and H mRNAs are generated compared to the N mRNA

than in acutely infected non-neural cells. However, this effect on the transcription gradient was only observed in three of the six cell lines tested (D-54, D-32 and U-251) indicating that there are cell specific factors that regulate transcription attenuation in the other cell lines. One such factor may be the interferon inducible protein MxA. After in vitro differentiation of cells by the treatment with papaverine (a membrane permeable analogue of cyclic AMP), cells such as D-54 and U-251 or TE671 (Miller and Carrigan, 1982), showed a selective reduction in the rate of synthesis of MV proteins. In the case of D-54 and U-251 cells the reduced synthesis was specific to viral mRNAs as general protein synthesis was not affected, nor was the translatability of the viral mRNAs affected in vitro. In general these studies showed that in cells of neuronal origin, cell specific factors and differentiation state may affect the expression of viral proteins. The relevance of these observations to mechanisms of in vivo persistence has not been determined.

5.3. Utilisation of "SSPE" derived viruses

It is important to distinguish between persistently infected cell cultures which have been established by co-culture of non-neural cell lines with brain material obtained from SSPE patients that harbours defective viruses such as the Biken virus in HEL cells and those set up after infection with so called "replication competent" SSPE isolates such as the Hallé, Lec, Mantooth and Horta-Barbosa virus strains. We have shown earlier that based on their similarity in gene sequences to the Edmonston strain the latter most likely represent laboratory contaminants (Rima et al., 1995) and as such these cultures are not further discussed here. A number of cell lines have been established by co-culture such as Lu106 cells, a human embryo lung fibroblast cell line persistently infected with a SSPE derived virus, the Biken–Vero cells used by the Hirano group for a number of studies of persistence mechanisms and a number of cell lines based on MA106 cells.

In the SSPE IP-3-Ca cell line Sheppard et al. (1985) demonstrated that the defect in viral budding was not due either to a decrease in the stability of the M gene mRNA or in the efficiency of its translation. Restriction occurred as a result of abnormally rapid post-translational degradation of the M protein. The mechanistic basis of this instability has never been established. Nevertheless, it provides a reasonable explanation for the defect in viral replication in the cells and it correlates with the lack of M expression noted earlier (Lin and Thormar, 1980). In contrast budding and fusion of the cells was reduced in HNT-Vero cells and Lu106 cells due to a restriction in F protein (Hummel et al., 1994; Sheshberadaran et al., 1985). In MA SSPE cells (Sheshberadaran et al., 1985), it was clear that it was a cellular factor that was important as these cells also failed to form syncytia on acute infection of the Edmonston virus in the cells. The Biken/HEL cell line that was derived from co-culture of SSPE brain tissue (Ueda et al., 1975) has been studied extensively. This persistent infection has been transferred into CV-1 cells by

co-culture. Sequencing analysis showed that all envelope-associated viral antigens had undergone mutations which led to the appearance of novel phenotypes. For example, in the H protein it was observed that haemadsorption was lost compared to the Nagahata strain, which is the wild-type precursor virus (Breschkin et al., 1979). Delayed activation of F_0 was not associated with mutations found at the furin cleavage site or the lack of the C terminal pentadecapeptide but appeared related to mutations in F_1 (Watanabe et al., 1995). However, the main emphasis has been on the study of the M protein which was shown to be unstable after translation and to have an eight amino-acid carboxy terminal extension and 20–22 mutations in the ORF (Ayata et al., 1989; Enami et al., 1989). The changes were later shown to be associated with biased hypermutation as they arose from uridine (U) to cytidine (C) mutations (Wong et al., 1991). The M protein has been shown not to have the normal RNP binding activity (Hirano et al., 1993). Reverse genetics was used to demonstrate that, when this M gene was inserted into the Edmonston genetic background, the resulting recombinant virus was infectious in CD46 transgenic mice following in intracerebral (i.c.) infection. The virus was also able to infect primary neuronal cultures from the transgenic mice in vitro (Patterson et al., 2001). Interestingly the Biken SSPE virus appears to out-compete wild-type MV in mixed infections of CV-1 (Hirano, 1992). Biken virus also out-competed the Edmonston strain better than the related Nagahata wild-type strain. Several mechanisms such as the presence of DI particles, or competition for interacting host or viral proteins were proposed to explain this. Nevertheless, it is clear that the resident Biken virus is at a selective advantage which could explain the accumulation of mutants and the replacement of wild-type viruses in SSPE. However, the mechanisms were unfortunately not further investigated.

Biken CV-1 cells do not normally form syncytia (Hirano et al., 1996). However, fusogenic c.p.e. was inducible by transfecting the cells with a plasmid DNA expressing human CD46. Replacing the transmembrane and cytoplasmic domains of CD46 with a glycosylphosphatidylinositol anchor did not prevent cell-to-cell fusion but abrogated the normal receptor down-regulation observed in persistently infected cells. The down-regulation of CD46 is determined by the presence of a so called "late domain" (YXXL) in the cytoplasmic domain of CD46 (Yant et al., 1997). These late domains govern interactions with cellular proteins that regulate degradation and trafficking of proteins within the cell. Mutations in CD46 that prevented down regulation enhanced syncytium formation indicating the role of the delicate balance between H and CD46 surface expression in controlling fusion in this system.

6. Persistence in the patient

SSPE has been shown to be caused by a persistent MV infection (Connolly et al., 1967, 1971). MV specific inclusions are present both in the cytoplasm and nuclei of infected cells (McQuaid et al., 1993). It is assumed that the intracytoplasmic inclusions represent the sites of transcription and replication. However, the nature of the nuclear inclusions has not been confirmed. These could be viral RNPs but are more likely to be complexes of viral N protein which has been shown to be able to translocate to the nucleus (Huber et al., 1991). The development of RT-PCR technology has allowed sequence analysis of amplicons derived from the defective viruses, which are hallmark of SSPE and MIBE. In general viral RNA is isolated at autopsy, but some samples have been derived from biopsy material. Most of the details of the mutations come from sequence analysis of persistent infections maintained by co-culture with monkey kidney cells or from direct sequence analysis of RNA extracted post mortem from the brain of the patients.

6.1. The defective nature of virus in SSPE and MIBE brains

Both SSPE and MIBE are characterised by mutations, which render the virus defective. These have been characterised in the 1980s and 1990s. The presence of mutations that may lead to defective budding are concentrated in the cytoplasmic tail of the F protein and in the M protein. Early on it was recognised that the hyperimmune response in SSPE to MV antigens was directed against all except the M protein. Defects in the levels of M protein are mediated by a number of mechanisms and mutations which affect the start codon, that make the protein unstable, enhance proteolytic degradation or lead to the generation of nonsense mutations, have all been identified (Cattaneo et al., 1986, 1989a,b; Hirano et al., 1993). In other instances some genes show clear evidence of so-called biased hypermutation in which U residues are changed to C residues (Cattaneo et al., 1988; Wong et al., 1989). The explanation that has been proposed to mediate this phenomenon involves the activity of double-stranded RNA-dependent adenosine deaminase (DRADA) which can act on double stranded (ds)RNA in replicative or transcriptive intermediates. DRADA has been shown to be present in enhanced levels in human IMR-32 neuroblastoma cell as compared to Vero cells (Rataul et al., 1992). However, it is not clear whether and when these dsRNA intermediates would arise in the normal infection cycle or if they are produced from collapsed transcription and/or replication processes. DRADA converts adenine to hypoxanthine and in subsequent replications this forms base pairs with U or C leading either to reversion or to a U $\rightarrow$ C mutation in the positive strand sequence, when dsRNA is generated transiently during transcription. Alternatively, if antigenome replication collapses an A $\rightarrow$ G mutation could result in the positive strand. The H gene of IP-3-CA SSPE virus is hypermutated and contains A $\rightarrow$ G mutations (Cattaneo et al., 1989a,b) and we have observed that by cloning a large number of M mRNAs from different areas of the brain of a particular SSPE patient (case B) that at least five distinct hypermutation events had taken place to give a hierar-

chy of more and more mutated M genes (Baczko et al., 1993). Whether the transcription of the M gene is different or if it is especially susceptible to biased hypermutation events is not known. It is more likely is that the process occurs frequently but that such events in other viral genes generate genomes that are unfit and counter-selected. There is only one paper reporting the possible presence of DI particles in SSPE brains (Sidhu et al., 1994). They have been shown to be present in measles vaccines (Bellocq et al., 1990) but it is unclear whether their presence affects the phenotype of the virus.

One further unexplored finding in MV persistence in cell culture and SSPE that is mirrored in cell culture studies into persistent infections using other paramyxoviruses, is a high degree of self-hybridisation when genome length RNA extracted from cultures or brain material was analysed (Hall and ter Meulen, 1976). This has been interpreted as indicative of a higher ratio of antigenome to genome RNP. The potential mechanism that underlies this observation, e.g. mutations in the promoters at genome ends have not been explored.

6.2. Altered transcription of measles virus in SSPE

Expression of the M protein can also be reduced by two transcriptional mechanisms. The first involves the generation of read-through transcripts of the P and M transcription units. In at least three SSPE patients, e.g. RNA derived from brain samples of case K (Cattaneo et al., 1986), and co-cultures with brain material that established the Yamagata and the MF cell lines (Cattaneo et al., 1987b) it was found that the RdRp was unable to recognise the signals in the GE sequences at the end of the P/V/C gene and GS sequences at the start of the M genes meaning that all M transcripts were in the form of P/M read-through RNAs. In these transcripts is unlikely that a scanning ribosome would get as far downstream as this to translate the third ORF and thereby synthesize M protein. Interestingly in both SSPE patients (cases K and MF), there were no changes in the GE, Ig and GS sequences and hence mutations leading to the altered properties of the transcriptase complex must lie elsewhere genome. In the case of the persistent infection with the Yamagata strain, a change in the intergenic sequence could explain the accumulation of the P/M read-through RNAs (Yoshikawa et al., 1990). Osaka viruses have well delineated changes in GS/GE sequences (Ayata et al., 2002).

The second transcriptional control mechanism affects the gradient of gene expression. The gradient varies from one host cell type to another and appears especially steep in MV infection in the human CNS. For example, in SSPE the gradient of gene expression is so steep that little F and H protein is produced, which may aid the virus in escaping detection by the immune system and would lead to a defect in budding (Cattaneo et al., 1987a,b). The reduction in transcripts of the L gene may or may not affect the level of viral RNA synthesis depending on whether the concentration of L protein is the rate-limiting step in transcription and/or replication. The effects of transcription attenuation could

be further enhanced if the respective promoter distal M, F and H mRNAs were less stable. However, to date there has been no evidence presented that reports a greater degree of instability of any specific MV mRNA. On the contrary, all data, although limited, so far argues for similar half lives of all viral mRNAs (Ogura et al., 1989).

Hyper-mutation and read-through transcription both lead to viruses that are defective in M protein expression. Recombinant viruses that have the M protein gene deleted are highly fusogenic and though they are able to penetrate the brain parenchyma of CD46-expressing interferon α/β receptor knockout mice deeply after i.c. infection, they cause less mortality (Cathomen et al., 1998b).

6.3. Mutations in the fusion protein in SSPE and MIBE

Other consistent alterations that occur in all SSPE cases lead to the loss of the carboxy-terminal pentadecapeptide of the F protein. This sequence is strictly conserved in all morbilliviruses and hence is assumed to have a specific, albeit as yet unknown function in viral budding. This region contains a tyrosine residue shown to be important as a basolateral sorting signal (Maisner et al., 1998) and it is also assumed that the cytoplasmic tail of F is involved in targeting the protein to lipid rafts and interaction with the M protein. This was confirmed by generating a recombinant MV which has the F and H proteins replaced by the G protein of vesicular stomatitis virus (Spielhofer et al., 1998). The resulting virions did not contain the M protein, illustrating that the protein is not absolutely required for virus assembly, although viral titres were significantly reduced. Viruses that lacked the F protein tail were found to be very fusogenic in cell culture. Cathomen et al. constructed recombinant viruses that had deletions in both the F and H tails and tested these in i.c. infection of transgenic CD46-expressing interferon α/β receptor knockout mice (Cathomen et al., 1998a). Similarly to viruses that expressed no M protein, these "tailless" mutants were found to be less lethal than the standard virus and to penetrate more deeply in the brain parenchyma (Cathomen et al., 1998b). Mutations that remove the conserved pentadecamer include the introduction of premature stop codons as well as frame shift mutations that lead to carboxyl-terminally extended or truncated F proteins. Deletion of a specific A nucleotide has been observed in a number of cases (Schmid et al., 1992). Mutants and wild-type sequences have been found in the same brain. The mechanism of this deletion is not known but it could be similar to that observed in human respiratory syncytial virus where deletions in stretches of A residues can alter the reading frame for the G protein leading to large scale differences in immunoreactivity of the protein (Garcia-Barreno et al., 1994).

6.4. Only wild-type virus sequences have been found in SSPE

Measles virus is a monotypic virus and the virus exists as a single serotype. Infection with one strain of measles appears

to provide life-long protection from the disease. With the development of RT-PCR and DNA sequencing techniques it has become clear that virus isolates vary in their nucleotide sequences especially in those encoding the last 150 amino acids of the N protein and the entire H protein (Rima et al., 1997; Rota et al., 1992, 1994; Taylor et al., 1991). During the last decade sequences of over 250 strains have been determined and these show that at least 21 genotypes exist which belong to eight different groups or clades. Although these viruses may once have been geographically restricted in their distribution, the increase in travel by children, who are the prime reservoir for virus replication, has now allowed worldwide distribution of most of the genotypes. Some of the clades appear to be extinct as no recent isolates have been made, but it is difficult to be certain about this as global measles surveillance is inadequate. In the USA and the UK, it has been possible to link the origin of the imported strain with travel by specific index cases (Rota et al., 2004). Thus the different genotypes have made it possible to establish transmission chains for a number of outbreaks.

The description of specific clades and genotypes of MV has allowed the evaluation of mutations found in the MV RNA sequences from SSPE brain material against wild-type (clades B–G) viruses. All the vaccine viruses are derived from the Edmonston strain (clade A) but no clade A virus has been found in SSPE brain material. The sequences found in SSPE brain are related to the wild-type viruses circulating at the time of initial infection of the child and not to those circulating at the time of onset of symptoms. Hence, the virus which initially infected the child, appears to persist and SSPE is not due to a super-infection by viruses circulating during the onset of symptoms (Jin et al., 2002; Rima et al., 1995; Rota, personal communication). To the best of the authors' knowledge no vaccine virus, genotype A, sequences have been obtained from SSPE cases. SSPE has been vastly reduced in incidence after successful control of measles by vaccination (Dyken et al., 1989). In contrast, vaccine strains have been identified in MV infections in immuno-compromised patients who died from MIBE (Bitnun et al., 1999) and giant cell pneumonia (Mawhinney et al., 1971).

7. In which tissues does measles virus reside before to onset of symptoms of SSPE?

Detection of MV RNA in the CNS of SSPE and MIBE cases is pathognomonic and without exception. However, the question has been raised as to where the virus persists in the patient prior to the onset of symptoms. Brown et al. (1989) documented finding MV in CNS and peripheral tissues obtained from SSPE patients by immunostaining for protein and in situ hybridisation for RNA. This study used the same in situ MV probe that was used by Fournier in his studies (Basle et al., 1986). It should be noted that this probe has been used extensively in both initial and ongoing in situ hybridisation studies of a variety of diseases, for example Paget's disease.

However, in none of the diseases have the results been confirmed by others and the fact that 70% of leukocytes were infected with MV in SSPE patients is at variance with all other data (Schneider-Schaulies et al., 1991). The most comprehensive search for the presence of MV virus outside the brain is reported in the Brown et al. study (1989). However, no clear picture emerges from this study as the findings vary between the cases to case and the results of in situ hybridisation and immunocytochemistry give are inconsistent. Several laboratories have examined other tissues isolated from SSPE patients and have not been able to find evidence of MV outside the brain. None of these studies have been reported in the literature due to the well-known bias against the publication of negative results. There is no question that SSPE is caused by persistent MV infection and it has been generally assumed that MV resides in the CNS before the onset of symptoms. However, there is no direct evidence to support this hypothesis. It would be difficult to obtain conclusive evidence either way, as these studies are made post mortem, hence represent late stages of the disease and therefore cannot answer questions about the site of persistence before the onset of symptoms. Once again this emphasises why in vitro studies in non-neural cell lines are still very useful. The fact that in MIBE there are all the hallmarks of MV hypermutation and spread of the virus over the entire brain but with a much shorter symptomless period between the acute infection and the manifestation of CNS infection could be seen as an argument against the persistence of MV in the CNS in SSPE cases. It appears that once the virus sets up neuronal infection it is difficult to stop and even though there is large variation in the length of time between onset of symptoms and death in SSPE, it is invariably fatal. Thus it is the level of the replication in the periphery that might be the controlling factor that determined the length of symptomless persistence. The alternative explanation may be that the virus has been in the CNS all along but has been controlled by the immune system. Until recently it was assumed that the CNS was subjected to no or at least lower levels of immune surveillance than many other organs and hence was a place where a virus such as measles could maintain itself, even though as an RNA virus it requires constant gene expression and low level replication in order to persist, which would, in any other tissue make it visible to the immune system. It is now clear that the CNS is under immune surveillance (McGavern et al., 2002). At one time the life-long immunity to measles virus demonstrated in Panum's island studies of 1846 was considered to be evidence for life-long persistence of the antigen (Panum, 1938). However, it is difficult to accept life-long persistence of measles virus RNA or protein in the absence of viral replication and low level gene expression. Indeed intracellular non-replicating MV nucleocapsids are inactivated within 3 days (Mottet et al., 1990). Hence the life-long immunity was considered evidence for viral persistence and SSPE cases might have been considered to occur whether the normal mechanisms to check this persistent infection had broken down. However, with the discovery of slow cycling memory
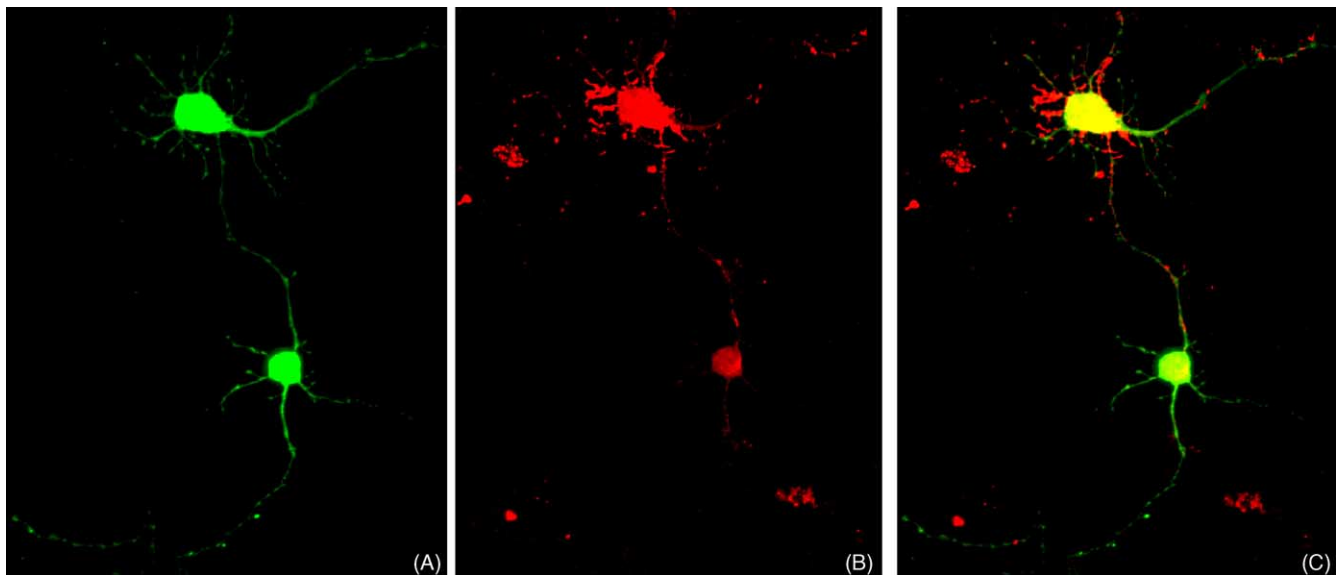
Fig. 3. Immunoreactivity and autofluorescence in primary mouse neurones infected with a recombinant measles virus which expresses EGFP and the H protein of a rodent-brain adapted strain of MV (CAM/RB). Cells were infected with the virus at a multiplicity of infection of 0.01. Cells were fixed using paraformaldehyde and examined by confocal scanning laser microscopy for autofluorescence and immunoreactivity. Photomicrographs represent an 8–10 μm composite optical section. Images were obtained in double excitation mode. The MV H glycoprotein (red) was detected and visualised by indirect immunofluorescence using a monoclonal antibody and a CY3 secondary antibody whilst EGFP (green) was detected by virtue of its autofluorescence. Two representative interconnected cells are shown. (A) EGFP autofluorescence, (B) H glycoprotein and (C) EGFP/H overlay. Magnification ×40.

CD8+ T cells it is no longer necessary to invoke the idea that life-long immunity equates to viral persistence (Wherry and Ahmed, 2004).

8. Is there a specific brain receptor for measles virus?

The role of the currently identified receptors in the spread of MV within the brain of SSPE patients is unclear. CD46 has not been detected on a differentiated neuronal cell line (McQuaid et al., 1998) and it has been shown that the virus can spread between contacting hippocampal neurones in the absence of CD46 expression and without extracellular virus production (Lawrence et al., 2000). The utility of recombinant viruses which express fluorescent proteins is evident in studies which aim to investigate the mechanism of MV cell-to-cell spread in vitro and a MV which expresses enhanced green fluorescent protein (EGFP) from an additional promoter proximal transcription unit has been used to infect astrocytoma, oligodendroglioma and neuroblastoma cell lines (Duprex et al., 1999b, 2000; Plumb et al., 2002). The recombinant MV (MVeGFP) has also been used to infect ex vivo hippocampal brain slices in which the virus was observed to spread unidirectionally, in a retrograde manner from CA1 to CA3 pyramidal cells and from there to the dentate gyrus (Ehrengrüber et al., 2002). MVeGFP has also been used to infect CD46-expressing interferon α/β receptor knockout transgenic mice by the i.c. route. Animals showed clinical signs of infection and EGFP was detected in discrete focal regions of the brain, in ependymal cells and neuroblasts and deep into the parenchyma (Duprex et al., 2000). Molecular determinants of neuropathogenesis have been mapped by generating a recombinant virus which expresses a rodent-brain adapted H glycoprotein in an Edmonston vaccine background (Duprex et al., 1999a). We have recently generated an equivalent virus which expresses EGFP (Duprex et al., unpublished) and have been able to infect dissociated non-transgenic mouse neurones in vitro. It is clear that this virus can spread transneuronally in the absence of CD46 in these cultures, reinforcing the suggestion that there are MV neuronal receptors. Furthermore, it is clear that although H is predominantly localised in the neuronal cell body it can also be detected in the long interconnecting axonal processes (Fig. 3). Collectively, these results suggest that the virus spreads transneuronally although this has not been formally proven. Recombinant viruses which expresses EGFP within the L protein (Duprex et al., 2002) or at the carboxy terminus of the P protein (Devaux and Cattaneo, 2004) of MV could help resolve this question as these viruses permit the visualisation of RNP.

9. Future perspectives

Forty years after the initial electron microscopy studies (Tellez-Nagel and Harter, 1966) and the identification of the link between MV and SSPE (Connolly et al., 1967; Payne et al., 1969) it is clear that much has been learnt about the plethora of alterations which occur in the genome which drive the virus to an intracellular existence. As such MV exemplifies long-term persistence of an RNA virus which

must continually replicate in the host and also evade immune detection. Nevertheless, many questions remain unanswered. Now that SLAM has been identified as a MV receptor, and since SLAM expression appears to be limited to dendritic cells and activated T and B lymphocytes as well as monocytes (Minagawa et al., 2001) two new questions arise. First: how does the virus transfer from the cells of the lymphoreticular system to the CNS, endothelia and epithelia? In vitro persistent infection models in SLAM expressing cells may help us gain understanding of this process. The second question is: how do cells of the lymphoreticular system enter the CNS? A Trojan horse mechanism has been proposed for the related morbillivirus canine distemper virus (Appel et al., 1981) and since it is now accepted that lymphocytes and macrophages traffic into the CNS, such a mechanism would no longer be controversial or problematic. The studies reviewed here also show that host factors play a role in the maintenance of MV persistence. In terms of CNS infection these may simply reflect the fact that neurons are less prone to generate innate immune responses so as to avoid cytotoxicity due to the generation of an inflammatory cytokine milieu in the CNS.

Some very basic questions also remain about the cell biology of a MV infection in the neuron in SSPE. If microfusion occurs at the synaptic membrane and if this involves an interaction between viral glycoproteins and receptors then what are the receptors and are both H and F involved; are there specific fusion receptors; how do the viral glycoproteins localise to the synaptic membrane; does this involve local protein synthesis, or are excretory vesicles containing viral glycoprotein transported to the synapses in a retrograde or anterograde pathway, using dynein or kinesin? It is unlikely that glycoproteins traffic from the cell membrane of neuron cell body along the axonal membranes to the synapse by simple diffusion. These questions are now answerable, at least in part by combined in situ hybridisation and immunocytochemistry, for cellular and viral proteins using multicolour fluorescent probes and viruses which express fluorescent proteins.

Finally, it is clear from the recent report on the generation of MIBE in a stem cell recipient (Freeman et al., 2004) that we need to take cognisance of the existence of persistent viruses when such medical interventions are performed. Another example of this is the potential difficulties porcine endogenous retroviruses (PERVs) may cause in xenotransplantation approaches (Magre et al., 2003). Indeed, widening this argument, it is clear that there are many viruses which have yet to be identified thus continuing to dissect the molecular mechanisms of MV persistence may help if and when such agents are identified.

10. Conclusions

Much has been learned about mutations that accumulate during persistence especially in SSPE but it has not been possible to assess the functional significance of these mutations. New approaches using reverse genetics will now allow us to introduce selective mutations in vaccine and wild-type viruses and to assess whether they alter the phenotype of the virus in such as a way as to aid persistence of the virus.

Acknowledgements

References

Anderton, P., Wild, T.F., Zwingelstein, G., 1981. Modification of the fatty acid composition of phospholipid in measles virus-persistently infected cells. Biochem. Biophys. Res. Commun. 103, 285–291.

Anderton, P., Wild, T.F., Zwingelstein, G., 1982. Phospholipids in a measles virus persistent infection: modification of fatty acid metabolism and fatty acid composition of released virus. J. Gen. Virol. 62, 249–258.

Anderton, P., Wild, T.F., Zwingelstein, G., 1983a. Accumulation of radiolabelled fatty acids in the neutral lipid fraction of measles virus persistently infected BGM cells. Biochem. Biophys. Res. Commun. 112, 29–34.

Anderton, P., Wild, T.F., Zwingelstein, G., 1983b. Measles-virus-persistent infection in BGM cells. Modification of the incorporation of [3H]arachidonic acid and [14C]stearic acid into lipids. Biochem. J. 214, 665–670.

Appel, M.J.G., Gibbs, E.P., Martin, S.J., ter Meulen, V., Rima, B.K., Stephenson, J.R., Taylor, W.P., 1981. Morbillivirus diseases of animals and man. In: Kurstak, I.V.E., Kurstak, C. (Eds.), Comparative Diagnosis of Viral Diseases. Academic Press, New York, pp. 235–300.

Ayata, M., Hirano, A., Wong, T.C., 1989. Structural defect linked to nonrandom mutations in the matrix gene of Biken strain subacute sclerosing panencephalitis virus defined by cDNA cloning and expression of chimeric genes. J. Virol. 63, 1162–1173.

Ayata, M., Komase, K., Shingai, M., Matsunaga, I., Katayama, Y., Ogura, H., 2002. Mutations affecting transcriptional termination in the p gene end of subacute sclerosing panencephalitis viruses. J. Virol. 76, 13062–13068.

Baczko, K., Lampe, J., Liebert, U.G., Brinckmann, U., ter Meulen, V., Pardowitz, I., Budka, H., Cosby, S.L., Isserte, S., Rima, B.K., 1993. Clonal expansion of hypermutated measles virus in a SSPE brain. Virology 197, 188–195.

Basle, M.F., Fournier, J.G., Rozenblatt, S., Rebel, A., Bouteille, M., 1986. Measles virus RNA detected in Paget's disease bone tissue by in situ hybridization. J. Gen. Virol. 67, 907–913.

Bellini, W., Englund, G., Rammohan, K., McFarlin, D.E., 1986. Evaluation of the structural proteins of the hamster neurotropic strain of measles virus with monoclonal antibodies. J. Neuroimmunol. 11, 149–163.

Bellocq, C., Mottet, G., Roux, L., 1990. Wide occurrence of measles virus subgenomic RNAs in attenuated live-virus vaccines. Biologicals 18, 337–343.

Bitnun, A., Shannon, P., Durward, A., Rota, P.A., Bellini, W.J., Graham, C., Wang, E., Ford-Jones, E.L., Cox, P., Becker, L., Fearon, M., Petric, M., Tellier, R., 1999. Measles inclusion-body encephalitis caused by the vaccine strain of measles virus. Clin. Infect. Dis. 29, 855–861.

Boriskin, Y., Bogomolova, N.N., Koptyaeva, I.B., Giraudon, P., Wild, T.F., 1986. Measles virus persistent infection: modification of the virus nucleocapsid protein. J. Gen. Virol. 67, 1979–1985.

Breschkin, A.M., Morgan, E.M., McKimm, J., Rapp, F., 1979. SSPE-BIKEN: a naturally arising hemagglutination-defective mutant of measles virus. J. Med. Virol. 4, 67–80.

Brown, H.R., Goller, N.L., Rudelli, R.D., Dymecki, J., Wisniewski, H.M., 1989. Postmortem detection of measles virus in non-neural tissues in subacute sclerosing panencephalitis. Ann. Neurol. 26, 263–268.

Burnstein, T., Jacobsen, L.B., Zeman, W., Chen, T.T., 1974. Persistent infection of BSC-1 cells by defective measles virus derived from subacute sclerosing panencephalitis. Infect. Immun. 10, 1378–1382.

Cathomen, T., Naim, H.Y., Cattaneo, R., 1998a. Measles viruses with altered envelope protein cytoplasmic tails gain cell fusion competence. J. Virol. 72, 1224–1234.

Cathomen, T., Mrkic, B., Spehner, D., Drillien, R., Naef, R., Pavlovic, J., Aguzzi, A., Billeter, M.A., Cattaneo, R., 1998b. A matrix-less measles virus is infectious and elicits extensive cell fusion: consequences for propagation in the brain. EMBO J. 17, 3899–3908.

Cattaneo, R., Rebmann, G., Baczko, K., ter Meulen, V., Billeter, M.A., 1987a. Altered ratios of measles virus transcripts in diseased human brains. Virology 160, 523–526.

Cattaneo, R., Rebmann, G., Schmid, A., Baczko, K., ter Meulen, V., Billeter, M.A., 1987b. Altered transcription of a defective measles virus genome derived from a diseased human brain. EMBO J. 6, 681–688.

Cattaneo, R., Schmid, A., Eschle, D., Baczko, K., ter Meulen, V., Billeter, M.A., 1988. Biased hypermutation and other genetic changes in defective measles viruses in human brain infections. Cell 55, 255–265.

Cattaneo, R., Schmid, A., Rebmann, G., Baczko, K., ter Meulen, V., Bellini, W.J., Rozenblatt, S., Billeter, M.A., 1986. Accumulated measles virus mutations in a case of subacute sclerosing panencephalitis: interrupted matrix protein reading frame and transcription alteration. Virology 154, 97–107.

Cattaneo, R., Schmid, A., Spielhofer, P., Kaelin, K., Baczko, K., ter Meulen, V., Pardowitz, J., Flanagan, S., Rima, B.K., Udem, S.A., 1989a. Mutated and hypermutated genes of persistent measles viruses which caused lethal human brain diseases. Virology 173, 415–425.

Cattaneo, R., Kaelin, K., Baczko, K., Billeter, M.A., 1989b. Measles virus editing provides an additional cysteine-rich protein. Cell 56, 759–764.

Celma, M.L., Fernandez-Muñoz, R., 1992. Measles virus gene expression in lytic and persistent infections of a human lymphoblastoid cell line. J. Gen. Virol. 73, 2203–2209.

Connolly, J.H., Allen, I.V., Hurwitz, L.J., Millar, J.H.D., 1967. Measles virus antibody and antigen in subacute sclerosing panencephalitis. Lancet 1, 542–544.

Connolly, J.H., Haire, M., Hadden, D.S., 1971. Measles immunoglobulins in subacute sclerosing panencephalitis. Br. Med. J. 1, 23–25.

Cremer, N.E., Oshiro, L.S., Hagens, S.J., 1979. Cyclic expression of antigen and infectious virus in a BHK cell line (0-853) persistently infected with an SSPE strain of measles virus. J. Gen. Virol. 42, 637–640.

Crespi, M., Chiu, M.N., Struthers, J.K., Schoub, B.D., Lyons, S.F., 1988. Effect of interferon on Vero cells persistently infected with Sendai virus compared to Vero cells persistently infected with SSPE virus. Arch. Virol. 98, 235–251.

Devaux, P., Cattaneo, R., 2004. Measles virus phosphoprotein gene products: conformational flexibility of the P/V protein amino-terminal domain and C protein infectivity factor function. J. Virol. 78, 11632–11640.

Dörig, R.E., Marcil, A., Chopra, A., Richardson, C.D., 1993. The human CD46 molecule is a receptor for measles virus (Edmonston strain). Cell 75, 295–305.

Duprex, W.P., Collins, F.M., Rima, B.K., 2002. Modulating the function of the measles virus RNA-dependent RNA polymerase by insertion of green fluorescent protein into the open reading frame. J. Virol. 76, 7322–7328.

Duprex, W.P., Duffy, I., McQuaid, S., Hamill, L., Cosby, S.L., Billeter, M.A., Schneider-Schaulies, J., ter Meulen, V., Rima, B.K., 1999a. The H gene of rodent brain-adapted measles virus confers neurovirulence to the Edmonston vaccine strain. J. Virol. 73, 6916–6922.

Duprex, W.P., McQuaid, S., Hangartner, L., Billeter, M.A., Rima, B.K., 1999b. Observation of measles virus cell-to-cell spread in astrocytoma cells by using a green fluorescent protein-expressing recombinant virus. J. Virol. 73, 9568–9575.

Duprex, W.P., McQuaid, S., Roscic-Mrkic, B., Cattaneo, R., McCallister, C., Rima, B.K., 2000. In vitro and in vivo infection of neural cells by a recombinant measles virus expressing enhanced green fluorescent protein. J. Virol. 74, 7972–7979.

Dyken, P.R., Cunningham, S.C., Ward, L.C., 1989. Changing character of subacute sclerosing panencephalitis in the United States. Pediatr. Neurol. 5, 339–341.

Ehrengrüber, M.U., Ehler, E., Billeter, M.A., Naim, H.Y., 2002. Measles virus spreads in rat hippocampal neurons by cell-to-cell contact and in a polarized fashion. J. Virol. 76, 5720–5728.

Enami, M., Sato, T.A., Sugiura, A., 1989. Matrix protein of cell-associated subacute sclerosing panencephalitis viruses. J. Gen. Virol. 70, 2191–2196.

Erlenhöfer, C., Wurzer, W.J., Loffler, S., Schneider-Schaulies, S., ter Meulen, V., Schneider-Schaulies, J., 2001. CD150 (SLAM) is a receptor for measles virus but is not involved in viral contact-mediated proliferation inhibition. J. Virol. 75, 4499–4505.

Fernandez-Muñoz, R., Celma, M.L., 1992. Measles virus from a long-term persistently infected human T lymphoblastoid cell line, in contrast to the cytocidal parental virus, establishes an immediate persistence in the original cell line. J. Gen. Virol. 73, 2195–2202.

Fishman, D., Wolfson, M., Bazarski, E., Segal, S., Rager-Zisman, B., 1997. The effects of measles virus persistent infection on AP-1 transcription factor binding in neuroblastoma cells. FEBS Lett. 410, 191–194.

Freeman, A.F., Jacobsohn, D.A., Shulman, S.T., Bellini, W.J., Jaggi, P., de Leon, G., Keating, G.F., Kim, F., Pachman, L.M., Kletzel, M., Duerst, R.E., 2004. A new complication of stem cell transplantation: measles inclusion body encephalitis. Pediatrics 114, 657–660.

Fujii, N., Kimura, K., Murakami, T., Indoh, T., Ishida, S., Fujinaga, K., Oguma, K., 1990. Suppression of interferon-induced oligo-2′,5′-adenylate synthetase induction in persistent infection. J. Gen. Virol. 71, 3071–3074.

Fujii, N., Oguma, K., Kimura, K., Yamashita, T., Ishida, S., Fujinaga, K., Yashiki, T., 1988. Oligo-2′,5′-adenylate synthetase activity in K562 cell lines persistently infected with measles or mumps virus. J. Gen. Virol. 69, 2085–2091.

Garcia-Barreno, B., Delgado, T., Melero, J.A., 1994. Oligo(A) sequences of human respiratory syncytial virus G protein gene: assessment of their genetic stability in frameshift mutants. J. Virol. 68, 5460–5468.

Gopas, J., Itzhaky, D., Segev, Y., Salzberg, S., Trink, B., Isakov, N., Rager-Zisman, B., 1992. Persistent measles virus infection enhances major histocompatibility complex class I expression and immunogenicity of murine neuroblastoma cells. Cancer Immunol. Immunother. 34, 313–320.

Gould, E.A., Linton, P.E., 1975. The production of a temperature-sensitive persistent measles virus infection. J. Gen. Virol. 28, 21–28.

Griffin, D.E., 2001. Measles virus. In: Knipe, D.M., Howley, P.M. (Eds.), Fields Virology, fourth ed. Lippincott, Williams and Wilkins, Philadelphia, PA, pp. 1401–1441.

Halbach, M., Koschel, K., 1979. Impairment of hormone dependent signal transfer by chronic SSPE virus infection. J. Gen. Virol. 42, 615–619.

Hall, W.W., ter Meulen, V., 1976. RNA homology between subacute sclerosing panencephalitis and measles viruses. Nature 264, 474–477.

Halsey, N.A., Modlin, J.F., Jabbour, J.T., Dubey, L., Eddins, D.L., Ludwig, D.D., 1980. Risk factors in subacute sclerosing panencephalitis: a case-control study. Am. J. Epidemiol. 111, 415–424.

Hirano, A., 1992. Subacute sclerosing panencephalitis virus dominantly interferes with replication of wild-type measles virus in a mixed infection: implication for viral persistence. J. Virol. 66, 1891–1898.

Hirano, A., Ayata, M., Wang, A.H., Wong, T.C., 1993. Functional analysis of matrix proteins expressed from cloned genes of measles virus variants that cause subacute sclerosing panencephalitis reveals a common defect in nucleocapsid binding. J. Virol. 67, 1848–1853.

Hirano, A., Yant, S., Iwata, K., Korte-Sarfaty, J., Seya, T., Nagasawa, S., Wong, T.C., 1996. Human cell receptor CD46 is down regulated through recognition of a membrane-proximal region of the cytoplasmic domain in persistent measles virus infection. J. Virol. 70, 6929–6936.

Huber, M., Cattaneo, R., Spielhofer, P., Orvell, C., Norrby, E., Messerli, M., Perriard, J.C., Billeter, M.A., 1991. Measles virus phosphoprotein retains the nucleocapsid protein in the cytoplasm. Virology 185, 299–308.

Hummel, K.B., Vanchiere, J.A., Bellini, W.J., 1994. Restriction of fusion protein mRNA as a mechanism of measles virus persistence. Virology 202, 665–672.

Jacobson, S., McFarland, H.F., 1982. Measles virus persistence in human lymphocytes: a role for virus-induced interferon. J. Gen. Virol. 63, 351–357.

Jin, L., Beard, S., Hunjan, R., Brown, D.W., Miller, E., 2002. Characterization of measles virus strains causing SSPE: a study of 11 cases. J. Neurovirol. 8, 335–344.

Joseph, B.S., Lampert, P.W., Oldstone, M.B., 1975. Replication and persistence of measles virus in defined subpopulations of human leukocytes. J. Virol. 16, 1638–1649.

Ju, G., Birrer, M., Udem, S., Bloom, B.R., 1980. Complementation analysis of measles virus mutants isolated from persistently infected lymphoblastoid cell lines. J. Virol. 33, 1004–1012.

Kirk, J., Zhou, A.L., McQuaid, S., Cosby, S.L., Allen, I.V., 1991. Cerebral endothelial cell infection by measles virus in subacute sclerosing panencephalitis: ultrastructural and in situ hybridization evidence. Neuropathol. Appl. Neurobiol. 17, 289–297.

Koschel, K., Muenzel, P., 1980. Persistent paramyxovirus infections and behaviour of beta-adrenergic receptors in C-6 rat glioma cells. J. Gen. Virol. 47, 513–517.

Lawrence, D.M., Patterson, C.E., Gales, T.L., D'Orazio, J.L., Vaughn, M.M., Rall, G.F., 2000. Measles virus spread between neurons requires cell contact but not CD46 expression, syncytium formation, or extracellular virus production. J. Virol. 74, 1908–1918.

Lin, F.H., Thormar, H., 1980. Absence of M protein in a cell-associated subacute sclerosing panencephalitis virus. Nature 285, 490–492.

Litvak, A.M., Sands, I.J., Gibel, H., 1943. Encephalitis complicating measles: report of fifty-six cases with followup studies in thirty-two. Am. J. Dis. Child 65, 265–295.

Ludlow, M., McQuaid, S., Cosby, S.L., Cattaneo, R., Rima, B.K., Duprex, W.P., 2005. Measles virus superinfection immunity and receptor redistribution in persistently infected NT2 cells. J. Gen. Virol., in press.

Lund, G.A., Tyrrell, D.L., Bradley, R.D., Scraba, D.G., 1984. The molecular length of measles virus RNA and the structural organization of measles nucleocapsids. J. Gen. Virol. 65, 1535–1542.

Magre, S., Takeuchi, Y., Bartosch, B., 2003. Xenotransplantation and pig endogenous retroviruses. Rev. Med. Virol. 13, 311–329.

Maisner, A., Klenk, H., Herrler, G., 1998. Polarized budding of measles virus is not determined by viral surface glycoproteins. J. Virol. 72, 5276–5278.

Maisner, A., Mrkic, B., Herrler, G., Moll, M., Billeter, M.A., Cattaneo, R., Klenk, H.D., 2000. Recombinant measles virus requiring an exogenous protease for activation of infectivity. J. Gen. Virol. 81, 441–449.

Mawhinney, H., Allen, I.V., Beare, J.M., Bridges, J.M., Connolly, J.H., Haire, M., Nevin, N.C., Neill, D.W., Hobbs, J.R., 1971. Dysgammaglobulinaemia complicated by disseminated measles. Br. Med. J. 2, 380–381.

McCullough, K.C., 1983. Characterization of a non-syncytiogenic autonomously replicating variant of measles virus. J. Gen. Virol. 64, 749–754.

McGavern, D.B., Homann, D., Oldstone, M.B., 2002. T cells in the central nervous system: the delicate balance between viral clearance and disease. J. Infect. Dis. 186 (Suppl. 2), S145–S151.

McQuaid, S., Campbell, S., Wallace, I.J., Kirk, J., Cosby, S.L., 1998. Measles virus infection and replication in undifferentiated and differentiated human neuronal cells in culture. J. Virol. 72, 5245–5250.

McQuaid, S., Kirk, J., Zhou, A.L., Allen, I.V., 1993. Measles virus infection of cells in perivascular infiltrates in the brain in subacute sclerosing panencephalitis: confirmation by non-radioactive in situ hybridization, immunocytochemistry and electron microscopy. Acta Neuropathol. (Berl.) 85, 154–158.

Meissner, N.N., Koschel, K., 1995. Downregulation of endothelin receptor mRNA synthesis in C6 rat astrocytoma cells by persistent measles virus and canine distemper virus infections. J. Virol. 69, 5191–5194.

Miller, C.A., Carrigan, D.R., 1982. Reversible repression and activation of measles virus infection in neural cells. Proc. Natl. Acad. Sci. U.S.A. 79, 1629–1633.

Minagawa, H., Tanaka, K., Ono, N., Tatsuo, H., Yanagi, Y., 2001. Induction of the measles virus receptor SLAM (CD150) on monocytes. J. Gen. Virol. 82, 2913–2917.

Minagawa, T., Sakuma, T., Kuwajima, S., Yamamoto, T.K., Iida, H., 1976. Characterization of measles viruses in establishment of persistent infections in human lymphoid cell line. J. Gen. Virol. 33, 361–379.

Mottet, G., Curran, J., Roux, L., 1990. Intracellular stability of nonreplicating paramyxovirus nucleocapsids. Virology 176, 1–7.

Moyer, S.A., Baker, S.C., Horikami, S.M., 1990. Host cell proteins required for measles virus reproduction. J. Gen. Virol. 71, 775–783.

Naniche, D., Varior-Krishnan, G., Cervoni, F., Wild, T.F., Rossi, B., Rabourdin-Combe, C., Gerlier, D., 1993. Human membrane cofactor protein (CD46) acts as a cellular receptor for measles virus. J. Virol. 67, 6025–6032.

Oglesbee, M.J., Kenney, H., Kenney, T., Krakowka, S., 1993. Enhanced production of morbillivirus gene-specific RNAs following induction of the cellular stress response in stable persistent infection. Virology 192, 556–567.

Ogura, H., Rima, B.K., Baczko, K., Nakamura, S., ter Meulen, V., 1989. Long-term effect of elevated temperatures on SSPE virus expression in persistently infected rat glial cells. Arch. Virol. 104, 259–269.

Panum, P., 1938. Observations made during the epidemic of measles on the Faroe Islands in the year 1846. Med. Classics 3, 829–886.

Patterson, J.B., Cornu, T.I., Redwine, J., Dales, S., Lewicki, H., Holz, A., Thomas, D., Billeter, M.A., Oldstone, M.B., 2001. Evidence that the hypermutated M protein of a subacute sclerosing panencephalitis measles virus actively contributes to the chronic progressive CNS disease. Virology 291, 215–225.

Payne, F.E., Baublis, J.V., Itabashi, H.H., 1969. Isolation of measles virus from cell cultures of brain from a patient with subacute sclerosing panencephalitis. N. Engl. J. Med. 181, 585.

Plumb, J., Duprex, W.P., Cameron, C.H., Richter-Landsberg, C., Talbot, P., McQuaid, S., 2002. Infection of human oligodendroglioma cells by a recombinant measles virus expressing enhanced green fluorescent protein. J. Neurovirol. 8, 24–34.

Radecke, F., Billeter, M.A., 1995. Appendix: measles virus antigenome and protein consensus sequences. Curr. Top. Microbiol. Immunol. 191, 181–192.

Radecke, F., Spielhofer, P., Schneider, H., Kaelin, K., Huber, M., Dotsch, C., Christiansen, G., Billeter, M.A., 1995. Rescue of measles viruses from cloned DNA. EMBO J. 14, 5773–5784.

Radecke, F., Billeter, M.A., 1996. The nonstructural C protein is not essential for multiplication of Edmonston B strain measles virus in cultured cells. Virology 217, 418–421.

Rager-Zisman, B., Egan, J.E., Kress, Y., Bloom, B.R., 1984. Isolation of cold-sensitive mutants of measles virus from persistently infected murine neuroblastoma cells. J. Virol. 51, 845–855.

Rager, M., Vongpunsawad, S., Duprex, W.P., Cattaneo, R., 2002. Polyploid measles virus with hexameric genome length. EMBO J. 21, 2364–2372.

Rapp, F., Robbins, S.J., 1981. Evolution of a human cell line persistently infected with measles virus. Intervirology 16, 149–159.

Rataul, S.M., Hirano, A., Wong, T.C., 1992. Irreversible modification of measles virus RNA in vitro by nuclear RNA-unwinding activity in human neuroblastoma cells. J. Virol. 66, 1769–1773.

Reddy, S.V., Menaa, C., Singer, F.R., Cundy, T., Cornish, J., Whyte, M.P., Roodman, G.D., 1999. Measles virus nucleocapsid transcript expression is not restricted to the osteoclast lineage in patients with Paget's disease of bone. Exp. Hematol. 27, 1528–1532.

Rice, J.M., Wolff, D.A., 1978. Establishment of a persistent measles virus infection in HEp-2 cells. Microbios 18, 179–188.

Rima, B.K., Davidson, W.B., Martin, S.J., 1977. The role of defective interfering particles in persistent infection of Vero cells by measles virus. J. Gen. Virol. 35, 89–97.

Rima, B.K., Earle, J.A., Baczko, K., ter Meulen, V., Liebert, U.G., Carstens, C., Carabana, J., Caballero, M., Celma, M.L., Fernandez-Muñoz, R., 1997. Sequence divergence of measles virus haemagglutinin during natural evolution and adaptation to cell culture. J. Gen. Virol. 78, 97–106.

Rima, B.K., Earle, J.A., Yeo, R.P., Herlihy, L., Baczko, K., ter Meulen, V., Carabana, J., Caballero, M., Celma, M.L, Fernandez-Muñoz, R., 1995. Temporal and geographical distribution of measles virus genotypes. J. Gen. Virol. 76, 1173–1180.

Rima, R.K., Martin, S.J., 1976. Persistent infection of tissue culture cells by RNA viruses. Med. Microbiol. Immunol. (Berl.) 162, 89–119.

Robbins, S.J., Pope, J.H., Sculley, T.B., 1991. Western blot analysis of antibody specificities in subacute sclerosing panencephalitis: reactivity to measles virus proteins produced in persistently infected cells. Intervirology 32, 52–58.

Rota, J.S., Hummel, K.B., Rota, P.A., Bellini, W.J., 1992. Genetic variability of the glycoprotein genes of current wild-type measles isolates. Virology 188, 135–142.

Rota, P.A., Bloom, A.E., Vanchiere, J.A., Bellini, W.J., 1994. Evolution of the nucleoprotein and matrix genes of wild-type strains of measles virus isolated from recent epidemics. Virology 198, 724–730.

Rota, P.A., Rota, J.S., Redd, S.B., Papania, M.J., Bellini, W.J., 2004. Genetic analysis of measles viruses isolated in the united states between 1989 and 2001: absence of an endemic genotype since 1994. J. Infect. Dis. 189 (Suppl. 1), 160–164.

Rustigian, R., 1962. A carrier state in HeLa cells with measles virus (Edmonston strain) apparently associated with non-infectious virus. Virology 16, 101–104.

Rustigian, R., 1966a. Persistent infection of cells in culture by measles virus. I. Development and characteristics of HeLa sublines persistently infected with complete virus. J. Bacteriol. 92, 1792–1804.

Rustigian, R., 1966b. Persistent infection of cells in culture by measles virus. II. Effect of measles antibody on persistently infected HeLa sublines and recovery of a HeLa clonal line persistently infected with incomplete virus. J. Bacteriol. 92, 1805–1811.

Rustigian, R., Winston, S.H., Darlington, R.W., 1979. Variable infection of Vero cells and homologous interference after co-cultivation with HeLa cells with persistent defective infection by Edmonston measles virus. Infect. Immun. 23, 775–786.

Schmid, A., Spielhofer, P., Cattaneo, R., Baczko, K., ter Meulen, V., Billeter, M.A., 1992. Subacute sclerosing panencephalitis is typically characterized by alterations in the fusion protein cytoplasmic domain of the persisting measles virus. Virology 188, 910–915.

Schneider-Schaulies, J., Dunster, L.M., Schneider-Schaulies, S., ter Meulen, V., 1995a. Pathogenetic aspects of measles virus infections. Vet. Microbiol. 44, 113–125.

Schneider-Schaulies, J., Schnorr, J.J., Brinckmann, U., Dunster, L.M., Baczko, K., Liebert, U.G., Schneider-Schaulies, S., ter Meulen, V., 1995b. Receptor usage and differential downregulation of CD46 by measles virus wild-type and vaccine strains. Proc. Natl. Acad. Sci. U.S.A. 92, 3943–3947.

Schneider-Schaulies, S., Kreth, H.W., Hofmann, G., Billeter, M., ter Meulen, V., 1991. Expression of measles virus RNA in peripheral blood mononuclear cells of patients with measles, SSPE, and autoimmune diseases. Virology 182, 703–711.

Schneider-Schaulies, J., Schneider-Schaulies, S., ter Meulen, V., 1993a. Differential induction of cytokines by primary and persistent measles virus infections in human glial cells. Virology 195, 219–228.

Schneider-Schaulies, S., Schneider-Schaulies, J., Bayer, M., Loffler, S., ter Meulen, V., 1993b. Spontaneous and differentiation-dependent regulation of measles virus gene expression in human glial cells. J. Virol. 67, 3375–3383.

Schneider-Schaulies, S., Schneider-Schaulies, J., Schuster, A., Bayer, M., Pavlovic, J., ter Meulen, V., 1994. Cell type-specific MxA-mediated inhibition of measles virus transcription in human brain cells. J. Virol. 68, 6910–6917.

Segev, Y., Ofir, R., Salzberg, S., Heller, A., Weinstein, Y., Isakov, N., Udem, S., Wolfson, M., Rager-Zisman, B., 1995. Tyrosine phosphorylation of measles virus nucleocapsid protein in persistently infected neuroblastoma cells. J. Virol. 69, 2480–2485.

Shaffer, J.A., Bellini, W.J., Rota, P.A., 2003. The C protein of measles virus inhibits the type I interferon response. Virology 315, 389–397.

Sheppard, R.D., Raine, C.S., Bornstein, M.B., Udem, S.A., 1985. Measles virus matrix protein synthesized in a subacute sclerosing panencephalitis cell line. Science 228, 1219–1221.

Sheshberadaran, H., Norrby, E., Rammohan, K.W., 1985. Monoclonal antibodies against five structural components of measles virus. II. Characterization of five cell lines persistently infected with measles virus. Arch. Virol. 83, 251–268.

Sidhu, M.S., Crowley, J., Lowenthal, A., Karcher, D., Menonna, J., Cook, S., Udem, S., Dowling, P., 1994. Defective measles virus in human subacute sclerosing panencephalitis brain. Virology 202, 631–641.

Smith-Jensen, T., Burgoon, M.P., Anthony, J., Kraus, H., Gilden, D.H., Owens, G.P., 2000. Comparison of immunoglobulin G heavy-chain sequences in MS and SSPE brains reveals an antigen-driven response. Neurology 54, 1227–1232.

Sorodoc, I., Aderca, I., Cajal, N., Dumitrescu, S.M., Constantinescu, O., Cernescu, C., 1980. Peculiarities of the virus-host cell relationship in a calf kidney cell line persistently infected with measles virus. Acta Virol. 24, 253–264.

Spielhofer, P., Bachi, T., Fehr, T., Christiansen, G., Cattaneo, R., Kaelin, K., Billeter, M.A., Naim, H.Y., 1998. Chimeric measles viruses with a foreign envelope. J. Virol. 72, 2150–2159.

Takasu, T., Mgone, J.M., Mgone, C.S., Miki, K., Komase, K., Namae, H., Saito, Y., Kokubun, Y., Nishimura, T., Kawanishi, R., Mizutani, T., Markus, T.J., Kono, J., Asuo, P.G., Alpers, M.P., 2003. A continuing high incidence of subacute sclerosing panencephalitis (SSPE) in the Eastern Highlands of Papua New Guinea. Epidemiol. Infect. 131, 887–898.

Tanaka, J., Nakamura, H., Fukada, T., 1987. Adult-onset subacute sclerosing panencephalitis: immunocytochemical and electron microscopic demonstration of the viral antigen. Clin. Neuropathol. 6, 30–37.

Tas, P.W., Koschel, K., 1991. Loss of the endothelin signal pathway in C6 rat glioma cells persistently infected with measles virus. Proc. Natl. Acad. Sci. U.S.A. 88, 6736–6739.

Tatsuo, H., Ono, N., Tanaka, K., Yanagi, Y., 2000. SLAM (CDw150) is a cellular receptor for measles virus. Nature 406, 893–897.

Taylor, M.J., Godfrey, E., Baczko, K., ter Meulen, V., Wild, T.F., Rima, B.K., 1991. Identification of several different lineages of measles virus. J. Gen. Virol. 72, 83–88.

Tellez-Nagel, I., Harter, D.H., 1966. Subacute sclerosing leukoencephalitis: ultrastructure of intranuclear and intracytoplasmic inclusions. Science 154, 899–901.

ter Meulen, V., Stephenson, J.R., Kreth, H.W., 1983. Subacute sclerosing panencephalitis. In: Fraenkel-Conrat, H., Wagner, R.R. (Eds.), Comprehensive Virology, vol. 18. Plenum Press, New York, pp. 105–159.

Ueda, S., Okuno, Y., Hamamoto, Y., Oya, H., 1975. Subacute sclerosing panencephalitis (SSPE): isolation of a defective variant of measles virus from brain obtained at autopsy. Biken. J. 18, 113–122.

Vainionpää, R., Salmi, A., Arstila, P., 1982. Characterization of a hamster brain cell line persistently infected with measles virus. Arch. Virol. 71, 311–322.

Valsamakis, A., Schneider, H., Auwaerter, P.G., Kaneshima, H., Billeter, M.A., Griffin, D.E., 1998. Recombinant measles viruses with mutations in the C, V, or F gene have altered growth phenotypes in vivo. J. Virol. 72, 7754–7761.

Watanabe, M., Wang, A., Sheng, J., Gombart, A.F., Ayata, M., Ueda, S., Hirano, A., Wong, T.C., 1995. Delayed activation of altered fusion glycoprotein in a chronic measles virus variant that causes subacute sclerosing panencephalitis. J. Neurovirol. 1, 412–423.

Wherry, E.J., Ahmed, R., 2004. Memory CD8 T-cell differentiation during viral infection. J. Virol. 78, 5535–5545.

Wild, T.F., Bernard, A., Greenland, T., 1981. Measles virus: evolution of a persistent infection in BGM cells. Arch. Virol. 67, 297–308.

Wild, T.F., Bernard, A., Malak, N.A., Brichon, G., Zwinglestein, G., 1986. Imprints of virus infection: can paramyxoviruses permanently modify triacylglycerol metabolism? Lipids 21, 608–611.

Wild, T.F., Dugre, R., 1978. Establishment and characterization of a subacute sclerosing panencephalitis (measles) virus persistent infection in BGM cells. J. Gen. Virol. 39, 113–124.

Wild, T.F., Malvoisin, E., Buckland, R., 1991. Measles virus: both the haemagglutinin and fusion glycoproteins are required for fusion. J. Gen. Virol. 72, 439–442.

Wolfson, M., Katorza, A., Segal, S., Rager-Zisman, B., 1989. Effect of persistent measles virus infection on protein kinase C activity and c-fos protooncogene expression in neuroblastoma cells. FEBS Lett. 250, 367–370.

Wong, T.C., Ayata, M., Hirano, A., Yoshikawa, Y., Tsuruoka, H., Yamanouchi, K., 1989. Generalized and localized biased hypermutation affecting the matrix gene of a measles virus strain that causes subacute sclerosing panencephalitis. J. Virol. 63, 5464–5468.

Wong, T.C., Ayata, M., Ueda, S., Hirano, A., 1991. Role of biased hypermutation in evolution of subacute sclerosing panencephalitis virus from progenitor acute measles virus. J. Virol. 65, 2191–2199.

Yant, S., Hirano, A., Wong, T.C., 1997. Identification of a cytoplasmic Tyr-X-X-Leu motif essential for down regulation of the human cell receptor CD46 in persistent measles virus infection. J. Virol. 71, 766–770.

Yoshikawa, Y., Tsuruoka, H., Matsumoto, M., Haga, T., Shioda, T., Shibuta, H., Sato, T.A., Yamanouchi, K., 1990. Molecular analysis of structural protein genes of the Yamagata-1 strain of defective subacute sclerosing panencephalitis virus. II. Nucleotide sequence of a cDNA corresponding to the P plus M dicistronic mRNA. Virus Genes 4, 151–161.

Young, K.K., Heineke, B.E., Wechsler, S.L., 1985. M protein instability and lack of H protein processing associated with nonproductive persistent infection of HeLa cells by measles virus. Virology 143, 536–545.